Contents

3.1 Introduction

The drug analysis training program provides preliminary training in basic technical areas.

The Center for Drugs and Evaluation Research (CDER) is responsible for establishing the inspectional and analytical guidelines associated with the FDA drug analysis programs. Drug Compliance Programs are found in the Compliance Program Guidance Manual (CPG).

This training chapter introduces the trainee to regulatory pharmaceutical analyses in FDA, analytical separation techniques, instrumentation, and the resolution to typical problems a trainee will encounter with pharmaceutical analyses. The trainee should discuss each exercise in advance with the trainer. If a laboratory does not perform analyses in that area, that portion of the training can be abbreviated or eliminated altogether.

The training is separated into modules using defined pharmaceuticals. Before beginning the sample analysis, the trainer should consider and discuss topics that will cultivate a thoughtful and responsible approach to each assignment. Topics may include the following:

- Why was the sample collected? (assume it was not a training sample).
- Criteria for method selection. What care is taken in selecting an analytical method? Is the method the correct one? Is the method valid?
- Correct sample handling. Is there a microbiological analysis associated with this sample that may necessitate the microbiologists to handle the sample first?
- Is there enough sample for all the regulatory tests? For an additional and check analysis? For an exhibit, if needed?
- What regulatory action might result from the analysis of the sample?

When the trainee has completed each exercise, the analysis, worksheet preparation, and answers to the questions should be discussed thoroughly. The analyst also shows that they are proficient in the technique and instrumentation.

3.2 Historical Background and Law

A. Introduction

The Food and Drug Act of June 30, 1906, prohibited adulteration and misbranding of drugs in interstate commerce. Following an adverse ruling (*United States v. Johnson*) by the Supreme Court of the United States in 1911, Congress passed the Sherley Amendment the same year, which added to the Act a prohibition against claims of curative or therapeutic effects being placed on the package label with intent to defraud the purchaser. The burden of proof of adequacy of claims, however, was on the government. Prior testing of drugs for safety was not addressed.

In early 1938, the Wheeler-Lea Amendment to the Federal Trade Commission (FTC) Act clarified the jurisdiction of advertising between the FTC and the FDA. The FTC reserved control of drug advertising. The weakening of FDA authority, in conjunction with the “Elixir of Sulfanilamide” drug-related disaster, paved the way for the passage of a completely new law.

The "Elixir of Sulfanilamide" tragedy of 1937 killed 107 people due to the use of the solvent diethyl glycol in the product. Since the law did not require prior testing of drugs for safety, there was no way to anticipate, or prevent, the marketing of this lethal mixture.

The FD&C Act was passed on June 25, 1938. The new law prohibited the marketing of "new drugs," (a term defined in the FD&C Act), unless the new drug had been tested and found to be safe for use under the prescribed conditions. The new law also required the active drug ingredient names and amounts be declared, and defined labeling be found on habit forming drugs. For false or fraudulent drug claims, the requirement that intent be demonstrated was dropped.

The Durham-Humphrey Amendment of 1951 defined the term "prescription drug"; these drugs could only be dispensed with a legal prescription. Over-the-counter (OTC) drugs were to bear labeling that contained directions for use, and warnings against misuse.

In 1962, the Thalidomide drug disaster, which demonstrated the danger of incomplete drug testing prior to clinical trials, ensured the passage of sweeping drug control legislation. Thalidomide, a sedative, taken during pregnancy, was found to cause the deformity known as phocomelia in children. The drug had never been approved for commercial sale in the United States. The use of this drug during pregnancy caused nine children in the United States to be born with this deformity. The tragedy had much greater proportions in Europe where thalidomide had been widely distributed.

Congress passed the Kefauver-Harris Drug Amendments on October 10, 1962, which answered the need for tighter controls over drugs. These amendments included the following changes:

- FDA was authorized to establish current good manufacturing practices (cGMPs) for drugs.
- Drug manufacturers were required to register annually.
- A drug is proven to be safe and effective by the manufacturer. A new drug could no longer be marketed prior to FDA approval based on convincing evidence of the drug's safety and effectiveness.
- Reasons were listed for which a previously approved drug could be removed from the market.
- New controls were placed on experimental and investigational drugs.
- Regulation of advertising of prescription drugs was returned to FDA.

In 1972, Congress passed the Drug Listing Act, which gave FDA the means to determine readily which drugs were actually being manufactured and commercially distributed.

B. Suggested Readings

1. "Requirements of Laws and Regulations Enforced by the U.S. Food and Drug Administration" (See Section 3.5 References 6), pp. 38-46

2. Chapter V of the FD&C Act (See Section 3.5 References 5)

3. Title 21 of the *Code of Federal Regulations*, Parts 201, 300, 314, and 369 (See Section 3.5 References 4) Found on the Gold Disk Web CD under Regulations.

4. Read the pertinent sections of "A Brief Legislative History of the Food, Drug, and Cosmetic Act" (See Section 3.5 References 11)

5. Chapter 1 of Bane's "Principles of Regulatory Drug Analysis" (See Section 3.5 References 2) and Part 1 of "A Chemist's Guide to Regulatory Drug Analysis" (See Section 3.5 References 3).

6. Compliance Policy Guidance Manual, Chapter 4, Subsection 400, Human Drugs (See Section 3.5 References 37).

3.3 Methodology

A. Introduction

Modern pharmaceutical analysis has evolved from partitioning an active ingredient into a volatile solvent and performing a titration or gravimetric analysis, to techniques that include almost every known analytical procedure. Spectrophotometry is performed in the three most accessible regions of the spectrum - the ultraviolet, visible, and infrared. Atomic absorption, fluorescence, flame emission, and mass spectrometry (GC/MS and LC/MS) are also used. Pharmaceutical analysis now relies heavily on chromatographic techniques, especially high performance liquid chromatography (HPLC), since these techniques provide rapid, accurate, qualitative, as well as quantitative analysis. These technique is also used to detect degradants and contaminants.

This section serves not only to review the methods, but more importantly, to understand where methods can be obtained for analyzing assigned drug samples. The analyst always considers two questions before beginning an analysis. Is the method the correct one? Is the method valid? This assignment will set the groundwork for answers to these questions.

B. Methodology Sources

1. Official Compendia

An "official compendium" is one that is officially cited in the FD&C Act (See Section 3.5 References 5). *The United States Pharmacopeia* (USP) (now combined with the *National Formulary* (NF)) is the major official compendium (See Section 3.5 References 7). The *Homeopathic Pharmacopoeia* is seldom used and is relatively obsolete. ORA Lab Manual, Volume IV, Section 1.3 Analytical Methods provides additional information on official compendia.

The USP, published annually, is citied in the Food Drug and Cosmetic Act, 201, (g)(1), 201(j), 501(b).

The USP contains valuable information. Read the following sections found in the Table of Contents: "Notices", "Monographs", "General", "Reagents"," Tables", "Index and the "Guide to General Chapters. The Guide to General Chapters contains general tests and assay information, scientific principles and theory, as well as drug regulations and laws.

The monograph, comprising a major part of the USP, serves as a definition and as a standard for a given drug substance, drug product, or drug ingredient. Read several monographs noting their organization and content.

It is recommended the analyst read these parts of the USP carefully and often.

2. Petition Methods (NDAs and ANDAs)

Each New Drug Application (NDA) (PAC 46.832) or Abbreviated New Drug Application (ANDA) (PAC 52.832)) includes a method for analysis of the final dosage form and in many cases the Active Pharmaceutical Ingredient (API). This is the control method used by the manufacturer, and it has been shown to be usable for regulatory purposes after testing by the FDA. Methods in manufacturer's Applications and Petitions that have been approved have "official" status. These include New Drug Applications (NDA), Abbreviated New Drug Applications (ANDA), New Animal Drug Applications (NADA), Food Additive Petitions (FAP), and Pesticide Petitions (PP).

All of the material in these NDA and ANDA submissions are confidential and subject to the protection required by the FD&C Act, sections 301(j) and 303(a) (5). The analyst may not cite NDA or ANDA methods in a publication, or discuss them with persons outside FDA. The documents require secure storage; the analysts are to be careful in how they handle the

documents. Further document security and confidentiality information can be found in the following : the *Supervisory Staff Manual Guides*, FDA F:2280.2, "Physical Security in Field Activities," Part 8, "Document Security", and 21 CFR Part 31.14 (See Section 3.5 References 4), "Confidentiality of Data and Information in a New Drug Application (NDA) File."

For more information about the Method Validation samples refer to Compliance Program CP7346.832 (most current version) and "Supplement to CP7346.832 Pre-approval Inspections/Investigations, 7/8/96."

3. AOAC Official Methods

The AOAC INTERNATIONAL (AOAC) *Official Methods of Analysis of the AOAC* (see Section 3.5 References 8) is specified in the *Code of Federal Regulations*, 21 CFR Part 2.19). Supplements, containing a cumulative index between editions, are published annually.

Sections to review in the AOAC include the following: "Definitions of Terms and Explanatory Notes," page xv, "Microchemical Methods," Chapters 18–22, Drugs I-V, and "Vitamins and Other Nutrients" During the review, note the presentation style and content found in these segments.

4. Pharmacopeial Forum

The *Pharmacopeial Forum* is a periodical issued six times a year by the United States Pharmacopeial Convention. It contains proposed new methods, updated lists of official reference standards and general comments on matters of interest in drug analysis and on use of the compendium.

5. Compliance Programs

Many Drug Compliance Programs, especially surveillance sample programs (PAC 56008) contain analytical directions, methods or modification of methods to analyze products. The analyst needs to review the compliance program prior to analysis.

6. Code of Federal Regulations

Title 21 of the *Code of Federal Regulations* (CFR) provides labeling requirements for a large number of pharmaceutical products. The CFR requirements frequently include official methodology to analyze the products. An example is Antacid Products for over-the-counter products found in 21CFR 331.

7. Journals and Other Literature

When methods are not located in the conventional sources mentioned above, there are applicable procedures found in the literature.

The *Laboratory Information Bulletin* (LIB) is a popular source of unofficial methodology in FDA published by the Division of Field Science. The LIBs are an excellent source for methods and resolution to problems the analyst may encounter with a method. Consult the latest LIB cumulative index to see if another FDA analyst has already solved the problem. If there is a related LIB method, it may be advantageous to discuss the problem with the author.

The *Journal of Pharmaceutical Sciences (J. Pharm. Sci.)* and the *Journal of AOAC INTERNATIONAL (J. AOAC Int.)* are useful references. An annual index is found in the last journal for the year.

International Pharmaceutical Abstracts, which contains abstracts from the two journals found above, is an excellent key to the entire pharmaceutical literature.

The *Analytical Profiles of Drug Substances/Analytical Profiles of Drug Substances and Excipients*, containing a series of monographs found in over thirty volumes, is another excellent reference. The first set of twenty volumes is edited by Klaus Florey of Squibb Institute; the remaining volumes are edited by Harry G. Brittain of Ohmeda Pharmaceutical Products Division, Inc., and published by Academic Press. A table of contents and cumulative index is included in each volume; approximately two volumes per year have been issued since 1996.

Other useful materials for completing the exercises that follow are given in Section 3.5 References (9-21).

8. Links to Scientific Sites:

www.acs.org: American Chemical Society. Find journals, articles of scientific interest, new trends in chemistry, and links to suppliers and vendors.

www.aoac.org: Association of Official Analytical Chemists. AOAC Publications, quality assurance issues, journal abstracts, approved method abstracts, and more.

www.ifpma.org: International Federation of Pharmaceutical Manufacturers Associations is a channel to exchange information within the international industry and various international organizations. Find International Conference on Harmonization (ICH) guidelines and related issues.

http://www.hazard.com/msds: Material Safety Data Sheets, Toxicology Reports, and other safety information.

http://medlib.cder.fda.gov/ CDER Medical Library web site includes e-journals, FDC Reports, and WebLERN site that includes the USP current version with monographs, American Drug Index, Physician's Drug Reference, Drug Facts and Comparisons, and other useful information.

C. Questions

1. What are the five most significant items of information in the USP "General Notices?" Compare the "General Notices" with the introductory chapter in the Official Methods.

2. Using the current USP, find the Dissolution procedure for Ascorbic Acid Tablets and how does it differ from most other Dissolution procedures? Look up the same monograph in USP 23. How does it differ and why was the change made?

3. Where in the USP is the listing for column types used in HPLC and GLC? How are they designated?

4. Locate the USP Reference Standards in the USP. What information is included here? What important information is missing and where is it found?

5. Learn how NDA and ANDA methods are filed and obtained in the laboratory.

6. Obtain an NDA method and review it as one might review a research paper. Is it complete, specific, and unambiguous? Does it meet the requirements of the Code of Federal Regulations?

7. What does the term "Official Compendium" mean? Name the three major types of "official" methods used by FDA.

3.4 Pharmaceutical Chemistry

The exercises that follow are presented as modules providing examples of the techniques needed to analyze a variety of pharmaceuticals in a variety of dosage forms according to the associated Program Assignment Code (PAC code).

3.4.1 Pharmaceutical Products Overview

The following outline serves as a guide in developing and customizing a drug training program by delineating pertinent knowledge areas and concepts with which a drug chemist needs to be familiar.

I. Modern Pharmacology
II. How a New Drug is developed
III. Standards (USP <11>)
    A. Working
    B. USP
    C. NIST
    D. Other Pharmacopeial Standards
IV. Controlled Substance--DEA Schedule
    Schedule I, II, III, IV, V (USP <1071>)
V. How Drugs Work - drugs and receptors
VI. Overview of the Chemistry of Organic Pharmaceuticals
    A. Basic Drugs and their Salts
    B. Acidic Drugs and their Salts
    C. Neutral Drugs
    D. Biotechnology Developed Drugs and Biopharmaceuticals (USP <1045>)
VII. Drug Formulations (USP 25 <1151> Pharmaceutical Dosage Forms)
    A. Tablets (immediate and extended release)
    B. Capsules (soft and hard gelatin)
    C. Liquid Products (solutions, elixirs)
    D. Topical Products (creams, ointments, gels, lotions)
    E. Injectionables (single and multidose)
    F. Transdermal patches and implants
    G. Other products (inhalation, aerosols, suppositories as listed in USP)
VIII. Types of Pharmaceuticals
    A. Analgesics
    B. Antianxiety - sedative, hypnotic
    C. Antiemetic
    D. Local anesthetics
    E. Antibiotics
    F. Antiviral
    G. Other
IX. Fillers, Excipients & Inert Materials used in Pharmaceuticals (USP <1074>)
    A. bulking materials and fillers
    B. lubricants
    C. colorants
X. Top 100 Commonly Prescribed Drugs and Narrow Therapeutic Range Drugs Lists
XII. Drug Programs and Sample Types in FDA
    A. Compliance Samples: PAC 56002
    B. Surveillance Samples: PAC 56008B
    C. New Drug Applications (NDA): PAC 46832
    D. Abbreviated New Drug Applications (ANDA): PAC 52832

E. Consumer Complaints: PAC 56R801
F. Shelf-Life Extension Program: PAC 88R_ _ _
G. Import Samples: PAC 56002
H. Method Validation Samples other than NDA/ANDAs
I. Other Program Samples: PAC 61003, 63001

A. Questions

1. When is a USP standard used and when is a used? What does one do when using a working standard that need not be done with USP standards and why?

2. What is the procedure in the laboratory for obtaining USP standards? NIST standards? Controlled drug standards?

3. Describe the differences between an immediate release tablet/capsule, an extended release tablet/capsule and a delayed release tablet. How would someone classify transdermal patches and implants?

4. What items would likely be found in compressed tablets and what are their purposes? In capsules? Name at least three items.

5. Describe to find where to find the PAC code for a product.

3.4.2 Instrumentation and Technique

3.4.2.1 Basic Analytical Techniques

A. Objective

To review and use very basic analytical techniques and equipment routinely used in the laboratory (listed below). The student is expected to read and become familiar with the following techniques as described in the USP. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

- Volumetric Glassware (care, usage and calibration) (USP <31>[volumetric apparatus] and <841> [Specific Gravity]),

- Balances and Weights (general, analytical, micro analytical) (USP <41>),

- Thermometers (mercury, alcohol, thermocouple) (USP <21>),

- pH (calibration and use) (USP <791>),

- Melting Range/Temperature (USP <741>),
- Gravimetric (USP <281> [Residue on ignition] and <271> [Readily Carbonizable Substances Test]),
- Titrations (aqueous and non-aqueous, pH, potentiometric),
- Water Determination (loss on drying and KF titration, USP <921>),
- Qualitative Chemical tests (USP <181>),
- Disintegration Test (USP <701>),
- Limits Tests (USP <231>[Heavy Metals]), and
- Uniformity of Dosage Units (USP <905>),

B. Exercises

Note: These tests may be performed in conjunction with other sub-sections of this section and need not be performed in order.

1. For the following exercises use product #1. 'Acetaminophen (API)' identified in Appendix 1.
   - Melting Range/Temperature (USP <741>), part 1.2,
   - Gravimetric (USP <281> [Residue on ignition] and <271> [Readily Carbonizable Substances Test]), parts 1.4 and 1.11,
   - Water Determination (Method I, USP <921>), part 1.3,
   - Qualitative Chemical tests (USP <181>), parts 1.5, 1.6, 1.7, 1.9, and 1.10, and
   - Limits Tests (USP <231> [Heavy Metals]), parts 1.8.
2. For the following exercises use product #2. 'Ascorbic Acid Tablets' identified in Appendix 1.
   - Titrations (aqueous) Assay part 2.1,
   - Disintegration Test (USP <701>) Use USP 23, part 2.3,

- Uniformity of Dosage (USP <905> [Weight Variation]), part 2.5, and
- Identification Tests A, B, C, part 2.2.

3. For the following exercises use product #6. 'Dextrose' identified in Appendix 1.
   - Water Determination (Method III) USP <921>, part 6.5,
   - Acidity - Titration (aqueous – limit test), part 6.4,
   - Identification – precipitate, part 6.1, and
   - Color of Solution - part 6.2.
4. Uniformity of Dosage Units <905> This test will be performed in other sub-sections of this section.
5. Many of the above tests will also be performed as part of the sample analysis for other sub-sections of this section. Appendix 1 also lists additional optional tests that may be included, especially if a full sample analysis is requested.

C. Questions

1. What other standards are used for determining the Melting Range and why are these used? What standards are used for calibration of the Apparatus? Why would someone not use the calibration standards for the analysis?
2. Why are limit and qualitative tests added to USP monographs? Compare the limit tests of the Acetaminophen product with those found in the Dextrose (including those not run). Which tests use the same method?
3. Both the Acetaminophen product and Dextrose have a method for Water. What is the difference between the two methods? Can the method used for Acetaminophen be used for Dextrose? Can the method used for Dextrose be used for Acetaminophen? Explain the answers.
4. Look at the USP general section on heavy metals <231>. What is the analyte of interest? Explain why Method I is used for Dextrose while Method II is used for Acetaminophen.

3.4.2.2 Spectrophotometric Techniques

Based on local need, each laboratory may choose any or all techniques to be presented to the student.

I. Ultraviolet/Visible Spectrophotometry (USP <851>)

A. Objective

To learn or review essentials of quantitative UV/VIS spectrophotometry and to operate spectrophotometers in the laboratory for drug analysis (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP or other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory

A. Essentials of spectrophotometry
   1. Ultraviolet Range
   2. Visible Range
   3. Near Infrared Range
B. Linearity
C. Limitations
D. Effects of solvent, pH and concentration

II. Instrumentation

A. Components:
   1. Source - Tungsten-halogen, Deuterium
   2. Monochromator – Single path, dual monochromator
   3. Sample compartment.- cuvettes (silica, glass, plastic)
   4. Detector
      - Photomultiplier
      - Diode & Diode Array
B. Calibration Procedures
C. Data Collection and Handling
   1. Data Collection Systems
   2. Evaluating Spectra and Data
   3. Qualitation
   4. Quantitation
   5. Data Storage

B. Exercises

1. Run holmium oxide filter and compare wavelengths obtained with listed values.

2. Run potassium dichromate solutions and compare to listed values.

3. Using several standard solutions from the drug exercise below, check the linearity of the instrument in a range of 0.1 AU to 2.0 AU.

4. For the following exercises use product 1, 'Acetaminophen (API)' identified in Appendix 1.

    - Identification Test B, UV <197U>, part 1.1, and

    - Assay – UV, part 1.13

5. For the following exercises use product 8. 'Reserpine and Hydrochlorothiazide Tablets' identified in Appendix 1. (Perform as part of Fluorometer training)

    - Diazotizable substances – colorimetric, part 11.4

C. Questions

1. Define: absorbance, absorptivity, molar absorptivity.

2. What are the typical cell size, specimen concentration and absorbance range used in the analysis of a substance in the UV or visible range?

3. What do the expressions "similar preparation" and similar solution" indicate (as used in tests and assays involving spectrophotometry in the USP)?

4. What do the expressions "concomitantly determine" and "concomitantly measured" indicate (as used in tests and assays involving spectrophotometry in the USP)?

5. Good practice demands that comparisons be made at the wavelength at which peak absorption occurs. What difference in nm for the wavelength specified in the USP monograph is considered acceptable?

II. Fourier Transform Infrared Spectrophotometry (FTIR) [USP <851>]

A. Objective

To review the theory, instrumentation and proper techniques for FTIR sample preparation and analysis in the drug laboratory (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP, other literature, or

through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory
   A. IR radiation
   B. Bonds
   C. Vibrations
   D. Resultant peaks

II. Instrumentation
   A. Prism
   B. Grating
   C. Fourier Transform

III. Sampling Techniques
   A. Nujol Mull
   B. KBr pellets
   C. Liquid cell

IV. FTIR instrument operation - Software –Programs, Macros
   A. Calibration
   B. Collect data
   C. Overlay
   D. Library Search
   E. Quantitation

V. Inspectional Aspects
   A. Calibration
   B. Quality of Spectra
   C. Accuracy

B. Exercises

1. Run a calibration curve on the instrument using a polystyrene film.

2. For the following exercises use product 1. 'Acetaminophen (API)' identified in Appendix 1.

   - Identification Test A, Infrared <197K>, part 1.1

3. Use the library function of the FTIR, if present, to compare spectra with that in the literature.

C. Questions

1. What is FTIR?

2. What is the advantage of an FTIR spectrometer (interferometer) over a conventional (dispersive) spectrometer (with a prism or grating monochromator)?

3. What is the purpose of the interferometer in the FTIR spectrometer?

4. List the types of sample preparation techniques used for analysis with an FTIR spectrometer and conventional/dispersive spectrometer, and explain when they would be used.

III. Fluorimetry [USP <851>] [includes Uniformity of Dosage Units <905>]

A. Objective

To review the theory, instrumentation and proper techniques for fluorometric sample preparation and analysis in the drug laboratory (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP, other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory
II. Instrumentation
    A. Excitation
    B. Emission
III. Sampling Techniques
IV. Operation
V. Quality Assurance

B. Exercises

1. For the following exercises use product #8. 'Reserpine and Hydrochlorothiazide Tablets' identified in Appendix 1.

    - Assay for reserpine – fluorescence, part 8.7,

    - Assay for hydrochlorothiazide – uv, part 8.8, and

    - Uniformity of Dosage Units <905>, part 8.4 & 8.5 (both reserpine and hydrochlorothiazide).

C. Questions

1. What is Fluorescence Spectrophotometry and compare it to conventional UV/Vis Spectrophotometry.

2. What advantages are seen in using fluorescence in this analysis? Disadvantages?

3. Why can't fluorescence be used for hydrochlorothiazide and why is fluorescence used for reserpine rather than UV?

4. How do the Assay results compare to the Average of the Uniformity of Dosage Units results? Explain why tablets may vary individually and why assay and the average of the UDU results may differ.

5. What does the USP expect if the 'Uniformity of Dosage Units' (USU) method differs from the Assay method? In the exercise above, perform the calculation (as a test). What percentage was found? When would this need a correction?

IV. Optical Rotation/Polarimeter [USP <781>]

A. Objective

To review the theory, instrumentation and proper techniques for optical rotation sample preparation and analysis in the drug laboratory (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP, other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory
II. Instrumentation
III. Sampling Techniques
IV. Operation of Instrument

B. Exercises

1. Check instrument calibration with solid phase calibrator.

2. For the following exercises use product 6. 'Dextrose' identified in Appendix 1.

   - Specific rotation, part 6.3, and

   - Assay (use procedure for Dextrose Injection), part 6.13

C. Questions

1. Describe polarimetry and the types of products for which it is used.

2. What is the purpose of the solid phase calibration cell and how is it used?

3. What is the general equation used in polarimetry and how does temperature effect polarimeter readings?

3.4.2.3 Chromatographic Techniques

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. The ultimate limits in efficiency for all chromatography (and all separations) are set by the rates of mass transport within the system. The system consists of several components.

I. Column Chromatography [USP <621>]

A. Objective

To acquaint the analyst with the underlying principles of column chromatography as a basis of all chromatographic techniques used for drug analysis (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP, other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory
II. Column Types
    A. Celite or Cellulose packed in Glass
    B. Solid Phase Extraction Columns (SPE) – Sep-Pak
    C. Gel
    D. Ion Exchange
III. Sampling Techniques

B. Exercises (Do one of the three depending on equipment located in the laboratory)

1. See Laboratory Information Bulletin (LIB) # 3357 and # 2571. Prepare a glass column packed with Solka-Floc material. Separate a color sample product such as grape juice or a mixed standard using the column and method cited. Collect each color and determine their identity using UV-Vis Spectrophotometry

2. See LIB # 3420 and # 2610. Separate a color sample product such as grape juice or a mixed standard using the column and method cited. Collect each color and determine their identity using UV-Vis Spectrophotometry.

3. For the following alternate exercises use product 5. 'Acetaminophen Oral Suspension' identified in Appendix 1. Use the USP XXII (1990) p. 13. 'Acetaminophen Oral Solution' Assay Procedure using a purified siliceous earth packed column.

C. Questions

1. When and why would someone use column chromatography?

2. (To be done at completion of each chromatography sections.). Prepare a chart, comparing and relating each of the techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy and quantitation.

II. Thin Layer Chromatography (TLC) [USP <621> and <201>]

A. Objective

To acquaint the analyst with the underlying principles of **T**hin **L**ayer **C**hromatography, (a form of planar chromatography) and the equipment and techniques used for presumptive identification/semi-quantitation of drug samples (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP or other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory
   A. Definitions of Terms
      1. Adsorbent
      2. Solvent front
      3. R_f value
      4. Origin
      5. Eluent
II. Types
   A. Silica Gel
      1. Binders
      2. Fluorescent Additives
   B. Cellulose
   C. High Performance (HPTLC)
III. Sampling Techniques
IV. Application Techniques

B. Exercises

1. For the following exercises use product 1. 'Acetaminophen (API)' identified in Appendix 1.
   - Identification Test C. <201>, part 1.1

2. Spot above sample or standard at several orders of magnitude of concentration. For example, 1ul, 2ul, 5ul, 10ul, 20ul, 50ul, 100ul, 200ul, and 500ul.

C. Questions

1. TLC is a qualitative method. How can someone use this technique as a semi-quantitative tool? As a quantitative tool?

2. The exercise uses UV light as a visualizing tool. What other visualizing tools are commonly used for TLC?

3. In exercise 2 above, what was the smallest spot seen? In the larger spots, were other 'breakdown' or 'related substances' spots seen in the chromatogram? If found, can this quantity be estimated based on the size of the smaller standard spots?

4. (To be done at completion of each chromatography sections). Prepare a chart, comparing and relating each of the techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling, and sampling techniques, automation, accuracy, and quantitation.

III. Gas Chromatography (GC) [USP <621>]

A. Objective

To present theory, instrumentation, parameters, and techniques for GC pharmaceutical analysis (topics outlined below). The student is expected to read and become familiar with the following techniques as described in the USP, other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory/Applications
II. Instrument Components
    A. Columns
        1. Packed Columns
        2. Capillary Columns
    B. Gases
    C. Temperatures
        1. Isothermal
        2. Temperature Program
    D. Injection
        1. Packed
        2. Split/Splitless
    E. Detectors
        1. FID
        2. ECD
        3. Nitrogen/Phosphorus
        4. MS
        5. Other

F. Data Handling
G. Calculation Techniques

III. Sampling Techniques
1. Direct Injection Techniques
2. Autoinjection
3. Headspace Sampling

IV. Operation

V. Quality Assurance (System Suitability)

B. Exercises

1. For the following exercises use product 5. 'Acetaminophen Oral Suspension' identified in Appendix 1.

   - Alcohol Content (if alcohol not already present spike at a 5% concentration) Method 2 <611> [packed column GC analysis], part 5.3

2. For the following exercises use product 1. 'Acetaminophen (API)' identified in Appendix 1. Prepare a series of standards.

   - Organic volatile impurities Method V <467> [Capillary column GC analysis], part 1.12

3. Calculate the results using both area and peak height calculations (electronically or manually) and compare results.

C. Questions

1. What type of products can be tested by GC? What products cannot be tested? Describe a GC method that can analyze products that normally cannot be tested by a GC method.

2. Describe how a Flame Ionization Detector works. Describe at least three other GC detectors commonly used. Which detector is similar to FID and how is it used?

3. Describe capillary GC and how it differs from packed column GC. What are the advantages and disadvantages of each technique?

4. When is it appropriate to use temperature programming? What are the advantages towards using temperature programming.

5. GC commonly uses four different gases. Air, Nitrogen, Helium and Hydrogen. What is the purpose of each gas, how is it used and at what flow rates?

6. (To be done at completion of each chromatography sections). Prepare a chart, comparing and relating each of the techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling, and sampling techniques, automation, accuracy, and quantitation.

IV. High Performance Liquid Chromatography (HPLC) [USP <621>]

A. Objective

To present theory, instrumentation, parameters, and techniques for HPLC pharmaceutical analyses (outline below). HPLC has become the most common and important analytical tool in the modern drug laboratory. The student is expected to read and become familiar with the following techniques as described in the USP or other literature, or through discussion with the trainer. The trainer is expected to provide explanations and devise simple exercises for the student to practice the techniques.

I. Theory/Application
II. Instrument Components
    A. Columns
        1. Normal Phase Columns
        2. Reverse Phase Columns
        3. Ion Exchange Columns
        4. Guard Columns
        5. FAST Columns
        6. Specialty Columns
    B. Column Parameters
        1. Packings
        2. Size (both column and packing)
        3. Dimensions
    C. Mobile Solvents
        1. Aqueous and non-aqueous
        2. Buffers
        3. PIC and ion-pair modifiers
        4. Solvent Programming
    D. Injection
        1. Techniques
        2. Autoinjection
    E. Solvent Delivery (Pumps)
        1. Isocratic
        2. Solvent Program
    F. Detectors
        1. UV
        2. Electrochemical

3. MS
4. Other Specialty

G. Data

III. Sampling Techniques

The need for clean samples and their preparation

IV. Quality Assurance (System Suitability)

B. Exercises

1. For the following exercises use product 5. 'Acetaminophen Oral Suspensions' identified in Appendix 1.

   - Assay. MeOH/H2O, Simple Isocratic systems, part 5.4, and
   - HPLC Identification Test B, and
   - pH <791> for product, parts 5.1 and 5.2.

2. For the following exercises use product 4. 'Acetaminophen and Caffeine Tablets' identified in Appendix 1.

   - Assay, HPLC multi-component with IS, part 4.4,
   - HPLC Identification, part 4.1, and
   - Dissolution and Uniformity of Dosage Units (HPLC), parts 4.2 and 4.3. (optional)

3. For the following exercises use product 3. 'Aspirin Tablets' identified in Appendix 1.

   - Assay, HPLC ion-pair system, part 3.5,
   - Limit of Free Salicylic Acid, HPLC, part 3.4,
   - Uniformity of Dosage Units (HPLC), Identification test A & B, (optional) and
   - Identification tests A & B. (Dissolution done as part of Dissolution exercise).

4. For the following exercises use product 7. 'Naltrexone Tablets' identified in Appendix 1.

   - Assay, HPLC gradient system, part 7.4,
   - Identification, part 7.1, and

- Optional – Uniformity of Dosage Units, part 7.3

C. Questions

1. Describe a typical 'basic' HPLC system and the purpose of each component.

2. What is the difference between normal phase and reverse phase? List at least three column types for each phase.

3. For the chromatograms obtained in exercise 2 'Acetaminophen and Caffeine Tablet' calculate the following for each of the peaks: Retention Time, Retention Volume, Relative Retention Time to IS, Capacity Factor, Resolution, Tailing, Theoretical Plates, Height Equivalent Theoretical Plate, Peak Widths at base, half height, and 5% height. Also, calculate one set of sample results using both peak area and peak height calculations. Are the results different? If so, explain why differences may result.

4. What is the purpose of the internal standard used in exercise 2? Calculate one set of sample results without using the internal standard. Are the results different? If so, explain why differences may result.

5. When and why would gradient elution be used in HPLC? What is the affect of temperature on HPLC? Would temperature programming such as found in GC be effective for HPLC?

6. (To be done at completion of each chromatography sections). Prepare a chart, comparing and relating each of the techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy, and quantitation.

3.4.2.4 Other Specialized Techniques

There are many specialized techniques used in pharmaceutical chemistry analysis. With the exception of dissolution, most of these techniques are unique to the specialty laboratory, or have not found wide recognition and thus are not usable for a basic training outline. All these techniques would be considered in advanced drug training. These other techniques will be mentioned by category only and could be expanded if the training laboratory has a need for this area of analytical techniques.

I. Dissolution: [USP <711>]

A. Objective

Dissolution is a technique commonly employed for tablet and capsule-type drug products. Modifications of the technique allow its use for other dosage forms such as patches, creams, and ointments. USP Apparatus 1 and 2 are covered in this section since these are the apparatus found in most drug laboratories.

I. Theory
II. Instrumentation
    A. USP Apparatus 1 (basket)
    B. USP Apparatus 2 (paddle)
    C. Other USP Apparatus (discussion only)
III. Calibration
    A. Mechanical Calibration
        1. Temperature
        2. Rotation Speed
        3. Vibration
        4. Height (positioning)
        5. Wobble
    B. USP Calibrators
        1. Dissolving (Prednisone)
        2. Non-dissolving (Salicylic Acid)
IV. Operation – Sampling
    A. Single Time period
    B. Profile Sampling
    C. Extended Release Products
V. Autosampling Techniques

B. Exercises

1. Calibrate an Apparatus 1 and an Apparatus 2 using the USP prednisone and salicylic acid calibrator tablets.

2. As part of the above calibration, perform a profile analysis for both prednisone and salicylic acid. Sample prednisone at 15-minute intervals for 1 hour then 30-minute intervals for an additional two hours. Sample salicylic acid at 30-minute intervals for two hours and at hourly intervals for an additional four hours.

3. For the following exercise use product #3. 'Aspirin Tablets' identified in Appendix 1.

    - Dissolution, Apparatus 1 @ 50-rpm --500ml pH 4.5 buffer – UV determination, part 3.3.

4. For the following exercise use product #4. 'Acetaminophen and Caffeine Tablets' identified in Appendix 1.

- Dissolution, Apparatus 2 @ 100-rpm – 900ml water – HPLC determination, part 4.2.

5. For the following exercise use product #2. 'Ascorbic Acid Tablets' identified in Appendix 1.

    - Dissolution, Apparatus 2 @ 50rpm –900ml water 'Procedure for pooled sample' – titration determination, Part 2.4.

C. Questions

1. What are the parameters that need be checked and corrected before dissolution can be run? Describe what effect each would have on an analysis.

2. What is the purpose of using two USP calibrators when checking a dissolution system?

3. Exercise 2 demonstrates a profile analysis. When would this technique be used? For what type of products could this technique be used? Look in the USP and list a monograph that uses a profile type analysis.

4. Describe the reason and procedure for removing air from the dissolution media.

5. Why not use water for all dissolution media and have constant paddle/basket rotation speed for all determinations?

6. Compare in-vitro dissolution results with in-vivo clinical studies? Explain the answer.

II. Advanced Techniques

To be developed as advanced training on an as-needed basis: (USP <___> identifies sections found in General Chapters, General Tests, and Assays found in the USP)

1. Particle Size Analysis (USP <786>)
2. Capillary Electrophoresis (CE)
3. Ion Chromatography
4. Atomic Absorption Spectrophotometry (AA) (USP <851>)
5. GC/Mass Spectrometry (USP <736>)
6. LC/Mass Spectrometry
7. Nuclear Magnetic Resonance (NMR) (USP <761>)
8. Refractive Index (USP <831>)
9. Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA) (USP <891>)
10. Polarographic Analysis (USP <801>)

11. Electrophoresis (USP <726>)
12. Immunoassays (USP <1045>)
13. Tablet Friability (USP <1216>)
14. Osmolarity (USP <785>)
15. Dissolution Apparatus III to VIII (USP <711> and <724>)
16. Drug Release (Extended Release and Delayed Release and Transdermal Systems) (USP <724>)
17. Analytical Biotechnology using CE, HPLC, Isoelectric focusing.

3.4.3 Inspections/Investigations

All analysts are to participate in at least one inspection prior to attending the FDA sponsored FDA Law and Evidence Development Course. In addition, analysts in drug specialty laboratories are provided specialized training in the inspection of pharmaceutical laboratories as defined in Current Good Manufacturing Practices (cGMPs) under 21CFR Parts 210 and 211. This training program is to be developed according to the needs of the laboratory. An example of training is as follows:

I. Types of Inspections
    A. GMP
    B. Pre Approval
    C. Post Approval
    D. Contract Laboratory
    E. Private Laboratory (Import work)
    F. Clinical Laboratory
    G. Foreign Inspections
II. Preparing for Team Inspections
III. How to start an inspection (482)
IV. What to look for in the Laboratory
V. Documentation
VI. Prepare a 483
VII. Prepare an EIR

3.5 References

The following is a general list of references. Some are FDA and other official source documents; these documents should be located in all laboratories. Some of the other listed references may not be found in all laboratory locations. Trainers and trainees should feel free to substitute additional and timelier materials.

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5. *Federal Food, Drug, and Cosmetic Act*, as amended. (1998, February). Washington, DC: U.S. Government Printing Office.

6. *Requirements of laws and regulations enforced by the U.S. Food and Drug Administration.* (1979). (HEW Publication (FDA) 79-1042, pp. 38-46.). DIANE Publishing Company.

7. *U.S. Pharmacopeia and National Formulary* (current ed.). Gaithersburg, MD: Association of Official Analytical Chemists International.

8. *AOAC official methods of analysis* (current ed.). Gaithersburg MD: Association of Official Analytical Chemists International.

9. *Remington's pharmaceutical sciences* (current ed.). Easton, PA: Mack Publishing Company.

10. *U.S. Pharmacopeia and National Formulary*. Selected portions of the Controlled Substances Act Regulations. Gaithersburg, MD: Association of Official Analytical Chemists International.

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19. Linsay, S. (1987). *High performance liquid chromatography, analytical chemistry by open learning*. New York: John Wiley & Sons.

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24. Snyder and Kirkland (1979). *Introduction to modern liquid chromatography* (2nd ed.) New York: Wiley Interscience.

25. Wilson, C. O. et al. (1971). *Textbook of organic medical and pharmaceutical chemistry* (6th ed., pp. 204-206). Philadelphia: Lippincott.

26. Ewing. (1971). *Instrumental methods of chemical analysis* (pp. 275-315).

27. Silverstein and Bassler. *Spectrometric identification of organic compounds*. New York: John Wiley & Sons.

28. George, B., McIntyre, P. (1987). *Infrared spectroscopy analytical chemistry by open learning*. New York: John Wiley & Sons.

29. Horvath and Nikelly. (1990). *Analytical biotechnology*. Washington, D.C.: American Chemical Society.

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31. Clark, G. C. (1975). *Isolation and identification of drugs* (Vol. 2). London: The Pharmaceutical Press. *British Pharmacopeia* (current ed.). London: Her Majesty's Stationery Office.

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3.6 Appendices

Appendix 1: Exercise Practice with Samples

Training Samples

Training samples may vary, and depend on what is located in the laboratory. The training samples, using USP products whenever possible, demonstrate the trainee's ability and

proficiency in performing regulatory tests. The following were used for the above training program and are examples of products that have been used successfully for training. However, other products may be used that demonstrate the same level of training. *Grayed parts of the following products were not used in the exercises but may be added to complete a full product analysis.* Full worksheets are to be generated for each product analyzed.

1. **Acetaminophen (API)** – USP, current edition, {Section 3.4.2.1B; 3.4.2.2 IB; 3.4.2.2 IIB; 3.4.2.3 IIB; 3.4.2.3 IIIB}
    - 1.1 Identification
        - Test A: Infrared <197K>
        - Test B: UV <197U>
        - Test C: TLC <201>
    - 1.2 Melting Range <741>
    - 1.3 Water Method 1 <921> KF Titration
    - 1.4 Residue on Ignition <281>
    - 1.5 Chloride <221> - limit test
    - 1.6 Sulfate <221> - limit test
    - 1.7 Sulfide – limit test
    - 1.8 Heavy Metals, Method II <231>
    - 1.9 Free p-aminophenol - colorimetric
    - 1.10 Limit of p-chloroacetanilide - TLC
    - 1.11 Readily carbonizable substances <271>
    - 1.12 Organic volatile impurities Method V <467> GLC – capillary column.
    - 1.13 Assay – UV

2. **Ascorbic Acid Tablets** – USP, current edition, { Section 3.4.2.1B, 3.4.2.4 IB}
    - 2.1 Assay – titration
    - 2.2 Identification – Test B
        - Test A – spot test
        - Test B – color test
        - Test C – color test
    - 2.3 Disintegration (Use USP 23 p. 129 procedure)
    - 2.4 Dissolution, Procedure for Pooled Sample <711>
    - 2.5 Uniformity of Dosage (Weight Variation)

3. **Aspirin Tablets** – USP, current edition { Section 3.4.2.3 IVB, 3.4.2.4 IB}
    - 3.1 Identification
        - Test A – color test
        - Test B – IR <197K>
    - 3.2 Dissolution <711> Apparatus 2 – UV Det
    - 3.3 Uniformity of Dosage Units <905>
    - 3.4 Limit of Free Salicylic Acid – HPLC
    - 3.5 Assay – HPLC with ion-pair reagent

4. **Acetaminophen and Caffeine Capsules** – USP, current edition, {Section 3.4.2.3 IVB, 3.4.2.4 IB}
    - 4.1 Identification - HPLC
    - 4.2 Dissolution <711> Apparatus 1 - HPLC
    - 4.3 Uniformity of Dosage Units <905> HPLC
    - 4.4 Assay – HPLC two component with IS

5. **Acetaminophen Oral Suspension** – USP, current edition,{Section 3.4.2.3 IB, 3.4.2.3 IIIB, 3.4.2.3 IVB}
    - 5.1 Identification – FTIR <197K>
        - A: HPLC
        - B: TLC <201>
    - 5.2 pH <791>
    - 5.3 Alcohol Content (if present) Method II <611>
        (if alcohol not present spike at 5% ethanol)
    - 5.4 Assay – HPLC

6. **Dextrose** – USP, current edition, {Section 3.4.2.1B, 3.4.2.2 IVB}
    - 6.1 Identification – color precipitate
    - 6.2 Color of Solution – limits test
    - 6.3 Specific Rotation <781S>
    - 6.4 Acidity – titration
    - 6.5 Water Method III <921> (drying)
    - 6.6 Residue on Ignition
    - 6.7 Chloride <221> - limit test
    - 6.8 Sulfate <221> - limit test
    - *6.9* Arsenic Method I <211> - limit test
    - 6.10 Heavy Metals <231> limit test
    - 6.11 Dextrin – limit test
    - 6.12 Soluble starch, sulfites – limit test
    - 6.13 Assay - Optical Rotation (Use procedure for Dextrose Injection, USP 24 p. 532).

7. **Naltrexone Tablets** – USP, current edition, { Section 3.4.2.3 IVB}
    - 7.1 Identification - HPLC
    - 7.2 Dissolution <711> Apparatus 2 – UV Determination.
    - 7.3 Uniformity of Dosage Units – HPLC gradient
    - 7.4 Assay – HPLC gradient

8. **Reserpine and Hydrochlorothiazide Tablets** – current edition {Section 3.4.2.2 IB, 3.4.2.2 IIIB}
    - 8.1 Identification
        - 8.1.1 Test A: UV
        - 8.1.2 Test B: TLC

8.2 Dissolution <711> Apparatus 2, reserpine – fluorescence
8.3 Dissolution <711> Apparatus 2, hydrochlorothiazide – uv.
8.4 Uniformity of Dosage Units - Content Uniformity for Reserpine – fluorescence
8.5 Uniformity of Dosage Units - Content Uniformity for Hydrochlorothiazide – U.V.
8.6 Diazotizable substances – colorimetric
8.7 Assay (Reserpine) – Fluorescence
8.8 Assay (Hydrochlorothiazide) – UV

Appendix 2: Statistical Analysis

Analytical results observed in the laboratory are to be accurate and reliable based upon statistical principles and by the current Good Manufacturing Practices. The following information is provided to ensure the status of the laboratory data in accordance with FDA promulgated regulations.

A. Definitions

Some of the definitions are from the ICH Validation Procedures definitions, which appeared in the Federal Register #60, March 1, 1995, p. 11260.

Number of Significant Places: The total number of digits, not counting leading zeros, from the first non-zero digit, to the last digit known with a reasonable confidence.

Error: Difference between the expected and the encountered value of some variable.

Incertitude: Lack of availability of information about a reading caused by the impossibility of reading closer than a certain readout limit. For example, a digital readout of 22.57, with no digits beyond the second decimal, has incertitude of +/- 0.01.

Standard Deviation, $\sigma = \sqrt{[\Sigma(x_i - x_{average})^2/(n-1)}$. See *Precision*.

RSD: Relative standard deviation. The standard deviation divided by the average; usually expressed as percent by multiplying it by 100.

Accuracy: The closeness of agreement between the value which is accepted (either as a conventional true value or as an accepted reference value) and the value found.

Precision: The closeness of agreement between a series of measurements obtained from multiple sampling under the same conditions. Usually expressed as standard deviation.

Repeatability: Precision measured in the same laboratory and under the same operating conditions, over a small time period.

Intermediate precision: Precision within the same laboratory but under different conditions, analysts and equipment.

Reproducibility: Precision between different laboratories, such as in collaborative studies.

Robustness: The ability of a procedure to remain unaffected by small but deliberate variations in method parameters. It is an indication of reliability during normal usage.

B. Using Significant Digits

Normally, the confidence in the value of a number decreases from the first to the last digit. Thus if a volume measurement gives 10.567 mL we may be quite confident that the value is close to 10 mL but less confident about the decimal "5", still less about the "6" and not really sure at all about the "7". In this case, the digit 7 is called the *least significant digit* (LSD). The number 10.567 is said to have 5 significant digits. The number of significant digits is obtained counting all digits that give reasonable confidence, not including leading zeros. For example, 0.0031 has two significant digits.

The rules of significant digits are rules of *data communication.* Only report the digits in which there is confidence. The non-significant ones are eliminated according to the rules outlined below. Data in process are kept with an extra significant figure and rounded to the correct significant figures for the final answer.. A good rule is using five digits throughout, but reporting at the end with the correct number of significant digits.

Note: Do not confuse significant digits, with *significant places*. The latter depend on the units used and the concept should be avoided in this context. For example 12.53 cm = 125.3 mm. Both have four significant digits, but different number of significant places (two and one respectively).

C. Performing Rounding

The procedure (see USP) for rejecting the digits beyond the least significant digit, LSD, is the following:

1. Establish the position of the LSD.

2. If the digit to the right of the LSD is less than 5, eliminate all remaining digits after the LSD.

3. If the next digit is larger than or equal to 5, increase the LSD by one and eliminate all the digits beyond the LSD.

Remember: The calculations are always retaining an extra significant figure over the significant ones. Rounding should be done only at the end to the correct significant figures.

D. Using Average and Standard Deviation

The average and standard deviation are estimates *after the fact*. This means that a number of measurements are first made and then their statistical properties are obtained from the analysis of the set of measurements. Calculations:

Average = $\Sigma(x_i)/n$, Sum of all data divided by the number of data

Variance = $\Sigma(x_i-x_{average})^2/(n-1)$ The number n-1 is the number of data minus one.

Standard deviation, $\sigma = \sqrt{[\Sigma(x_i-x_{average})^2/(n-1)}$

%RSD = Relative Standard Deviation = (standard deviation)/(average)*100

See ORA Lab Manual, Volume III, Section 7.0, Statistics for further information

3.7 Answer Key

3.3 Methodology

1. **What are the five most significant items of information in the USP "General Notices?" Compare the "General Notices" with the introductory chapter in the Official Methods.** This is a subjective answer but the answer could include the following: The use of "Official" and "Official Article"; Significant Figures and Tolerances; Reference Standards/Reagents; Tests and Assays; Preservation, Packaging, Storage and Labeling (especially storage temperature); Weights and Measures; and Concentrations. The "Guide to General Chapters" contains information regarding procedures used throughout the USP; the General Notices serves as a general guidance document.

2. **Using the current USP, find the Dissolution procedure for Ascorbic Acid Tablets and how does it differ from most other Dissolution procedures? Look up the same monograph in USP 23. How does it differ and why was the change made?** Ascorbic Acid Tablets uses a pooled sample procedure where equal volumes of each vessel are pooled and the one-pooled solution is assayed. The assay procedure is performed on the solution from each vessel for most Dissolution procedures. The USP 23 uses the Disintegration test that measures the time that a tablet fully disintegrates and is only a physical test. The test did not measure the concentration of the ascorbic acid actually in solution.

3. **Where in the USP are the listings for column types used in HPLC and GLC? How are they designated?** The column types for both HPLC and GLC is listed in USP section <621> Chromatography under Chromatography Reagent. HPLC is listed as *packings*

with a letter designation beginning with 'L'. GLC is listed as *phases* with a letter designation beginning with 'G'.

4. **Locate the USP Reference Standards in the USP. What information is included here? What important information is missing and where is it found?** The USP Standards are found in section <11> and generally list only name, use, and storage conditions. The lot number, amount supplied, and the cost for each standard are found in the most recent issue of the *Pharmacopeial Forum* published bimonthly by the U.S. Pharmacopeia. The same information can be also located in the most recent issue of the USP Reference Standards Catalog or found on-line at www.usp.org.

5. **Learn how NDA and ANDA methods are filed and obtained in the laboratory.** The answer to this question will depend on the District and Laboratory Policy; the trainee is to describe the laboratory procedure.

6. **Obtain an NDA method and review it as one might review a research paper. Is it complete specific, complete, and unambiguous? Does it meet the requirements of the Code of Federal Regulations?** The answer to this will depend on the NDA selected. The trainee's answer is to be reviewed and critiqued by an experienced analyst familiar with NDAs and ANDAs. Trainee should reference 21 CFR part 201 (Labeling), 310 (New Drugs), and 314 (Applications for FDA Approval to Market a New Drug).

7. **What does the term "Official Compendium" mean? Name the three major types of "official" methods used by FDA.** "The term 'official compendium' means the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, official National Formulary, or any supplement to any of them." See Section 3.5 References (5) Chapter II Definitions Sec. 201 (j), three major types of "official" methods used by FDA are the (1) United States Pharmacopeia, (2) AOAC Official Methods. (3) FDA approved New Drug Application or Abbreviated New Drug Application methods.

3.4.1 Pharmaceutical Products Overview

1. **When is a USP standard used and when is a working standard used? What does one do when using a working standard that does not need to be done with USP standards and why?** The USP standard is to be used for the analysis of all USP products. Working standards are used for products and dosage forms not found in the USP. The purity and identity of all working standards are to be verified before use to assure that the standard is sufficiently pure and it is the correct material. The standard purity is to be used in the assay calculations. The USP standard is assumed to be 100% unless otherwise started on the label.

2. **What is the procedure in the laboratory for obtaining USP standards? NIST standards? Controlled drug standards?** The answer to this question is dependent on the

District and Laboratory Policy. The trainee is to describe this procedure and reference the Laboratory's SOP.

3. **Describe the differences between an immediate release tablet/capsule, an extended release tablet/capsule and a delayed release tablet. How would one classify transdermal patches and implants?** Immediate release tablet and capsules release the active ingredient within a small period of time, typically less than 30-minutes. Extended Release tablets release the active ingredient at a sustained and controlled release rate over a period of time. Typically extended release tablets and capsules release their ingredient with time periods of 8 hours, 12 hours, 16 hours, and 24 hours. Delayed Release tablets release the pharmaceutical dosage after a set time. These are frequently enteric coated to prevent release in the stomach thus release the dosage in the intestinal track. Transdermal patches and implants would be classified as extended release products, but release the dosage over a much greater period of time such as weeks or months.

4. **What items would likely be found in compressed tablets and what is their purpose? In capsules? Name at least three items.**
   - Active Ingredient – supplies pharmacological dose: any drug product.
   - Diluents (Fillers) – provide bulk: lactose, calcium phosphate, microcrystalline cellulose.
   - Binders – impart cohesive properties: starch, povidone (PVP).
   - Lubicants – reduce mechanical friction and prevent tablets from sticking to punches and dies: magnesium stearate.
   - Glidants (Flow Agents) – promotes free flowing characteristics: colloidal silicon dioxide, talc.
   - Disintegrants – enable tablets to break apart in aqueous environments: starch, crospovidone (PVP-XL), croscarmellose, Na starch glycolate.
   - Capsule – holder for active and filler materials: gelatin.

5. **Describe to find where to find the PAC code for a product** All FDA collected samples appear in FACTS, which lists the PAC codes and the type of sample. PACs for the various programs are located in the Compliance Program Guidance Manual (CPGM). The CPGM frequently provides additional sample information including methodology to be used for the products being analyzed.

3.4.2.1 Basic Analytical Techniques

1. **What other standards are used for determining the Melting Range and why are these used? What standards are used for calibration of the Apparatus? Why would someone not use the calibration standards for the analysis?** A USP Reference Standard or National Formulary Reference Standard corresponding to an authentic specimen of the substance being tested is added in equal parts to the substance until an intimate mixture is obtained. Agreement of observations on the authentic and the mixture provides reliable

evidence of chemical identity. USP Melting Point Reference Standards are used for calibration of the apparatus. There are six such standards: Acetanilide, Caffeine, Phenacetin, Sulfanilamide, Sulfapyridine and Vanillin. The one that melts nearest the expected melting temperature of the substance under test is used.

2. **Why are limit and qualitative tests added to USP monographs? Compare the limit tests of the Acetaminophen product with those found in the Dextrose (including those not run). Which tests use the same method?** USP monographs serve to define the identity, strength, quality, and purity of the substances for which they are written. Limit and qualitative tests are part of that determination. Tests for the presence of foreign substances and impurities are provided to limit such substances to amounts that are unobjectionable. Both monographs use the same tests for residue on ignition <281>, chloride <221>, and sulfate <221>. Acetaminophen also has limit tests for water (<921>Method I), sulfide, heavy metals (<231>Method II), readily carbonizable substances <271>, free p-aminophenol, and p-chloroacetanilide. Dextrose has limit tests for water (<921>Method III), Arsenic (<211>Method I), heavy metals (231), dextrin and soluble starch sulfites.

3. **Both the Acetaminophen product and Dextrose have a method for Water. What is the difference between the two methods? Can the method used for Acetaminophen be use for Dextrose? Can the method used for Dextrose be used for Acetaminophen? Explain the answers.** The method used for water determination in Acetaminophen is titrimetric. There is a quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer, which reacts with hydrogen ions (Karl Fischer Reagent – sulfur dioxide & iodine dissolved in pyridine & methanol). The procedure is typically performed as a residual titration where excess Reagent is added, and after a sufficient time has passed, to allow for complete reaction. Unconsumed reagent is then titrated with a standard solution of water in a solvent such as methanol. The residual procedure avoids difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly. A gravimetric method is used for water determination in Dextrose. The Dextrose is dried at 105 degrees for 16 hours. The hydrous form loses between 7.5% and 9.5% of its weight. The anhydrous form loses not more than 0.5% of its weight. The method for Acetaminophen and Dextrose are not interchangeable because one has to account for the water of hydration. This cannot be accurately done for dextrose using the oven drying technique.

4. **Look at the USP general section on heavy metals <231>. What is the analyte of interest? Explain why Method I is used for Dextrose while Method II is used for Acetaminophen.** The test is used to determine the content of metallic impurities that are colored by sulfide ion, specifically lead (percentage by weight). The substance being tested is visually compared to a control Standard Lead Solution. The sample is calculated as total Heavy Metals equivalent to lead. Method I is used for substances that yield clear/colorless preparations under the specified test conditions. Method II is used when the resulting preparation is not clear/colorless or for substances that interfere with the precipitation of

metals by sulfide ion or for fixed and volatile oils. In Method II, sample is initially introduced into a crucible and charred.

3.4.2.2(I) Ultraviolet/Visible Spectrophotometry

1. Define: absorbance, absorptivity, molar absorptivity.

Beers Law supplies the terms: $\log_{10}(1/T) = A = abc$

- Absorbance: [A] is the logarithm, to the base 10, of the reciprocal of the transmittance (T).
- Absorptivity : [a] – the quotient of the absorbance (A) divided by the product of the concentration of the substance (c) expressed in grams per liter, and the absorption path length (b) in cm.
- Molar Absorptivity: [Symbol like a rounded capital E] -- the quotient of the absorbance (A) divided by the product of the concentration, expressed in moles per liter, of the substance and the absorption path length in cm. It is also the product of the absorptivity (a) and the molecular weight of the substance.

2. What are the typical cell size, specimen concentration and absorbance range used in the analysis of a substance in the UV or visible range?

- Typical cell size (path) is 1 cm.
- For many pharmaceutical substances 'concentrations of about 10 ug of the specimen per ml often will produce absorbances of 0.2 to 0.8 in the ultraviolet or the visible region.' Ref. USP <851>.

3. What do the expressions "similar preparation" and "similar solution" indicate (as used in tests and assays involving spectrophotometry in the USP)? The expressions "similar preparation" and "similar solution" indicate that the reference specimen, generally a USP Reference Standard, is to be prepared and observed in a manner identical for all practical purposes to that of the test specimen. Ref. USP <851>.

4. What do the expressions "concomitantly determine" and "concomitantly measured," indicate (as used in tests and assays involving spectrophotometry in the USP)? The expressions "concomitantly determine" and "concomitantly measured" indicate that the absorbances of both the solution containing the test specimen and the solution containing the reference specimen, relative to the corresponding test blank, are to be measured in immediate succession. Ref. *USP <851>*.

5. Good practice demands that comparisons be made at the wavelength at which peak absorption occurs. What difference in nm for the wavelength specified in the USP monograph is considered acceptable? Should this differ by more than +/- 1 nm from the

wavelength specified in the individual monograph, re-calibration of the instrument may be indicated. Ref. USP <851>.

3.4.2.2(II) Fourier Transform Infrared Spectrophotometry (FTIR)

1. **What is FTIR?** A computerized process in which the source of energy is sent through an interferometer (instead of a monochromator) and onto a sample which is irradiated. An interferogram (intensity vs. time spectrum) is obtained, which has the characteristics of a spectrum. A computer mathematically converts the (intensity vs. time) spectrum (fourier transform) into an (intensity vs. frequency) fourier transform infrared spectrum.

2. **What is the advantage of an FTIR spectrometer (interferometer) over a conventional (dispersive) spectrometer (with a prism or grating monochromator)?**

H. Conventional/Dispersive	Interferometer
• A large number of moving parts • Requires 7 1/2 minutes to 30-minutes for one scan • Slow scan speed • To improve resolution adjust slits • No internal reference for frequency accuracy • Stray light within the instrument • Thermal problems because sample is close to IR source • IR radiation by sample is viewed by the detector	• Only the mirror is in motion • Produces a spectrum in as little as 1 second • Rapid scan speed • No slits in the system to define resolution • Laser provides an internal calibration system with frequency accuracy • No equivalent to stray light • Sample further removed from the IR source • Emitted radiation by the sample is not viewed by the detector

3. **What is the purpose of the interferometer in the FTIR spectrometer?** The interferometer replaces the conventional monochromator so that energy throughput is inherently greater (no slits) and all wavelengths present reach the detector during the entire time needed to observe a spectrum. The interferometer is coupled to a detector and a computer that reduces the data to an ordinary spectral curve faster.

4. **List the types of sample preparation techniques used for analysis with an FTIR spectrometer and conventional/dispersive spectrometer, and explain when they would be used.**

- Mulls formed by grinding the compound and dispersing it in mineral oil (nujol). The oil/sample mixture is then placed between salt plates and pressed together.
    - Poor solubility in a useful solvent and saving of time in sampling handling.
- Gases and vapors in gas sample cells produce spectra, which differ fundamentally from those observed in a condensed state (solids, liquids, solutions).
    - Sample is to be in a gaseous state that can be transferred to a gas sample cell.
- Solutions by dissolving the compound (liquid or solid) in a solvent and placing it in a sealed liquid cell.
    - Sealed cell is perhaps the simplest technique to use for liquids with viscosities less than or similar to water.
- KBr discs (pellets) prepared using a press.
    - A dried powdered or crystalline sample is ground with KBr.

3.4.2.2(III) Fluorometry

1. **What is Fluorescence Spectrophotometry and compare it to conventional UV/Vis Spectrophotometry.**
    - "Fluorescence Spectrophotometry is the measurement of the emission of light from a chemical substance while it is being exposed to ultraviolet, visible, or other electromagnetic radiation. In general, the light emitted by a fluorescent solution is of maximum intensity at a wavelength longer than that of the excited radiation, usually by some 20 to 30 nm."
    - "Fluorescence Spectrophotometry is often more sensitive than absorption spectrophotometryabsorption spectrophotometry. In absorption measurements, the specimen transmittance is compared to that of the blank; and at low concentrations, both solutions give high signals. Conversely, in fluorescence spectrophotometry, the solvent blank has low rather than high output, so that the background radiation that may interfere with determinations at low concentrations is much less. Whereas few compounds can be determined conveniently at concentrations below 10-5M by light absorption, it is not unusual to employ concentrations of 10-7M to 10-8M in fluorescence spectrophotometry." Ref. USP<851>.

2. **What advantages are seen in using fluorescence in this analysis? Disadvantages?**
    - Only reserpine fluoresces – hydrochlorothiazide does not. Also the sensitivity of the fluorescent solution is much higher than found in the UV of reserpine thus interferences from other products are minimized.
    - Fluorescence does not have the accuracy as UV and is subject to quenching. The overall disadvantage in the analysis is that two different solutions and preparations need be prepared with the reserpine requiring an additional nitrite step and different instruments need be used.

3. **Why can't fluorescence be used for hydrochlorothiazide and why is fluorescence used for reserpine rather than UV?**
    - Hydrochlorothiazide does not fluoresce.
    - Hydrochlorothiazide is not easily separated from the reserpine and would interfere with the quantative UV determination of reserpine.

4. **How does the Assay results compare to the Average of the Uniformity of Dosage Units results? Explain why tablets may vary individually and why assay and the average of the UDU results may differ.**

    The answer to this question depends on the results, however since the UDU procedure is very similar to the Assay procedure then results should be reasonably close.
    - Tablets are manufactured as heterogeneous mixtures. Thus small variations will occur between individual tablets due to the manufacturing processes (from minor variations in die settings and formulation mixtures, etc.).
    - The Assay and UDU result may vary. The UDU analyzes each tablet whereas the Assay is a composite that includes both statistically high and low tablets. Look at the range and standard deviation of the UDU. If the standard deviation is high then the UDU average versus the assay results may have a larger difference.

5. **What does the USP call for if the 'Uniformity of Dosage Units' method differs from the Assay method? In the exercise above, perform the calculation (as a test). What percentage was found? When would this need a correction?** The USP calls for a special 'correction of the results.' The procedure requires calls furan analysis of a composite of the sample equivalent to one tablet and compare this value with that of the average value for UDU. The difference is calculated as a correction factor "F..." If "F" differs by more than 3%, then each UDU value needs to be corrected to reflect the change due to the procedural differences. This answer calls for a calculation obtained from the analysis.

3.4.2.2(IV) Optical Rotation/Polarimeter

1. **Describe polarimetry and the types of products for which it is used.** Polarimetry is the measurement of optical rotation. Many pharmaceutical substances are optically active in the sense that they rotate an incident plane of polarized light so that the transmitted light emerges at a measurable angle to the plane of the incident light. Ref. USP<781>. Pharmaceutical products that show a dextrorotatory, or (+) optical isomers, and levorotatory, or (-) optical isomer. The products that show such optical rotatory power are chiral.

2. **What is the purpose of the solid phase calibration cell and how is it used?** The solid phase calibration cell is used for checking the calibration of the polarimeter. This calibrator consists of a plate of quartz mounted in a holder perpendicular to the light path. The calibrator standard readings are traceable to NIST. The cell replaces the need to prepare Dextrose and Sucrose solutions that also can be used for calibration. The solid phase

calibration cell is stabilized to a temperature of 25°C and placed in the instruments cell holder. The degree of rotation of the cell can be read directly on the instruments readout system.

3. **What is the general equation used in polarimetry and how does temperature effect polarimeter readings?** The general equation for polarimetry is $[\alpha]t/\lambda$ where 'α' is specific rotation at 't' temperature / λ wavelength, which equals 100a/lc where 'a' is the observer rotation in degrees, 'l' is the path length in decimeters and 'c' is the concentration in grams per 100 ml. Temperature is in the equation and has a direct effect on the measured specific rotation. Temperatures need to be maintained within 0.5°C of the stated value in the USP for the calculations to be valid for a measured product.

3.4.2.3(I) Column Chromatography

1. **When and why would someone use column chromatography?** Column chromatography offers a wide choice of stationary phases and is useful for the separation of individual compounds, in quantity, from mixtures." Ref. USP <621>. This technique is used for color analysis and when large quantities of purified materials are needed.

2. (**To be done at the completion of each chromatography sections.). Prepare a chart, comparing and relating each of techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy, and quantitation.** *See Q & A Attachment A*

3.4.2.3(II) Thin Layer Chromatography (TLC)

1. **TLC is a qualitative method. How can someone use this technique as a semi-quantitative tool? As a quantitative tool?** A visual comparison of the size of the spots may serve for semi-quantitative estimation. Quantitative measurements are possible by means of densitometry, fluorescence, and fluorescence quenching; or the spots may be carefully removed from the plate, followed by elution with an effective solvent and spectrophotometric measurement. Ref. USP <621>.

2. **The exercise uses UV light as a visualizing tool. What other visualizing tools are commonly used for TLC?** The spots produced by paper or thin-layer chromatography may be located by: (1) direct inspection if the compounds are visible under white or either short-wavelength (254 nm) or long-wavelength (360 nm) UV light; (2) inspection in white or UV light after treatment with reagents that will make the spots visible. Ref. USP <621>.

3. **In exercise 2 above, what was the smallest spot seen? In the larger spots, were other 'breakdown' or 'related substances' spots seen in the chromatogram? If found, can this quantity be estimated based on the size of the smaller standard spots?** (Result from

experiment reported here.) Most of the USP monographs that use the TLC technique for chromatographic purity call for preparing a reference solution with a known concentration, usually a low concentration. For example, if a standard of about 2ug is prepared and a breakdown spot is observed, an analyst can estimate the quantity as "NMT 2 ug" if the R_f and the intensity of the secondary spot are close to the values of the reference standard solution.

4. **(To be done at completion of each chromatography sections.). Prepare a chart, comparing and relating each of techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy, and quantitation. See Q & A Attachment A**

3.4.2.3(III) Gas Chromatography (GC)

1. **What type of products can be tested by GC? What products cannot be tested? Describe a GC method that can analyze products that normally cannot be tested by a GC method.** GC is a chromatographic technique that can be used on any pharmaceutical product that contains volatile organic compounds of interest. Typical tests may include assay, impurity testing and organic volatile impurity analysis. The product is to be volatile above the lower temperature limit of the stationary phase and below the upper limit of the stationary phase. For non-volatile compounds (for example sugars) and thermally labile compounds (for example antibiotics) derivatization can be used to transform normally non-volatile or thermally unstable compounds to a volatile compound usable for GC.

2. **Describe how a Flame Ionization Detector works. Describe at least three other GC detectors commonly used? Which detector is similar to FID and how is it used?**
    - A FID detector consists of an air/hydrogen flame and a collector electrode. Ions collected produce an electrical signal.
    - Electron-Capture detector (contains a radioactive source of ionized radiation) is very sensitive for compounds containing halogens and nitro groups.
    - Thermal Conductivity detector (employs a heated wire placed in the carrier gas stream) is used for all volatile compounds but has very low sensitivity. This is sometimes considered to be the universal detector since it can detect most volatile compounds regardless of structure.
    - Nitogen-Phosphorus or alkali flame-ionization detectors (contains a thermionic source such as alkali-metal salt or rubidium glass bead) are very sensitive for organic nitrogen or phosphorus compounds but have low sensitivity for other hydrocarbons.
    - Mass Spectrometer detector is used to identify ion fragments of the parent molecule. This is a very sensitive detector that can give molecular structural information for the compound of interest.
    - The Nitrogen Phosphorus Detector is a variant of the FID. If the same sample and column are used with an FID and NPD, the same chromatogram will be produced

except the NPD will ignore the hydrocarbon solvent and other non-nitrogen containing components and produce a much simpler chromatogram. Used for nitrogen containing drugs.

3. **Describe capillary GC and how it differs from packed column GC. What are the advantages and disadvantages of each technique?**
    - Capillary GC columns are long (25 - 50 meters), thin (0.18 to .53mm ID) fused silica columns with higher separation efficiency (more theoretical plates) and superior resolution than that of a packed GC column (typically 2 meters long and ¼ inch diameter).
    - Advantages are their high efficiency and sturdiness. They are easy to handle and do not break as easily as glass columns. The capillary column is widely used for complex mixtures because of their superior resolution
    - The disadvantages of capillary GC is that the columns can become overloaded, which affects the chromatography and it needs a modified inlet or injection port. Capillary are also generally more expensive.

4. **When is it appropriate to use temperature programming? What are the advantages towards using temperature programming?** If there is a wide range of boiling points, the analyst needs to increase the column temperature over time in order to elute the high boiling point compounds of interest. Besides decreasing the time needed for analysis it provides improved peak shapes, and because later peaks are now taller and sharper, it provides better detection.

5. **GC commonly uses four different gases. Air, Nitrogen, Helium and Hydrogen. What is the purpose of each gas, how is it used and at what flow rates?**
    - Air is typically used as the oxidant to support the flame in a FID detector. Typical flows are 350 – 450 mls/min.
    - Nitrogen and Helium are typically used as carrier gases. The type of carrier gas used is dependent on the detector used. For capillary and megabore columns, typical flows are 0.5 – 10mls/min, for packed columns 8 – 40 mls/min. Nitrogen and helium can also be used as make-up gas in an FID detector when using capillary columns. Make-up gas + column flow in a FID detector typically should be 25- 30mls/min.
    - Hydrogen is typically used as the fuel to support the flame in a FID detector. Typical flow rates are 30 ml/min. It is also sometimes used as a carrier gas due of its high efficiency in temperature programming as per the van Deemter curve.

6. **(To be done at completion of each chromatography sections.). Prepare a chart, comparing and relating each of techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy, and quantitation. See Q & A Attachment A**

3.4.2.3(IV) High Performance Liquid Chromatography (HPLC)

1. Describe a typical 'basic' HPLC system and the purpose of each component.

A typical liquid chromatograph consists of:

- Reservoir - to contain the mobile phase.
- Solvent Delivery or Pumping System – causes the mobile phase to flow through the system at a specified flow rate. Single or multi-pump designs are can be purchased.
- Injector - to introduce the sample into the mobile phase. Autosamplers are commonly used.
- Column - component in which the separation is achieved by different mechanisms such as partition, adsorption, or ion-exchange of compounds in the test solution between the mobile and stationary phases.
- Detector – detects or measures compounds as they elute from a column. Common detectors include Single Wavelength UV, Diode Array/UV, Refractive Index and Fluorescence.
- Data collection device - to receive and store detector output – usually a computer or integrator.

2. What is the difference between normal phase and reverse phase? List at least three column types for each phase.

- Systems consisting of polar stationary phases and nonpolar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and non-polar stationary phases, and are called reverse-phase chromatography.
- Examples of column type for each phase:
  Reversed Phase: C18, C12, C8, C4, C2, C1, Phenyl
  Normal Phase: Silica, CN, NH_2, PAC, Diol, Alumina

3. For the chromatograms obtained in exercise 2 'Acetaminophen and Caffeine Tablet' calculate the following for each of the peaks: Retention Time, Retention Volume, Relative Retention Time to IS, Capacity Factor, Resolution, Tailing, Theoretical Plates, Height Equivalent Theoretical Plate, Peak Widths at base, half height, and 5% height. Also calculate one set of sample results using both peak area and peak height calculations. Are the results different? If so explain why differences may result.

(Formulas and calculations to be shown) Example given below: (Student is to perform exercise using information obtained from experiment.)

HPLC Exercise

Note: Electronic integrators perform most of the following calculations in automated systems. However, it is important to verify if the formula used by the system conforms to the USP

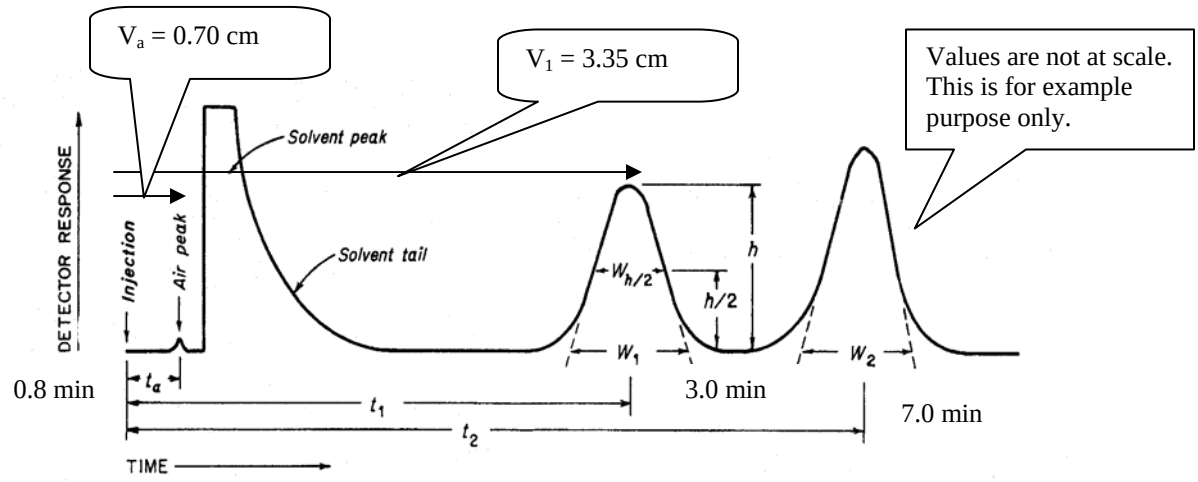

Figure 1: Chromatographic separation of two substances

In which V_a and V_1 are the retention volumes for the non-retained component and the compound under test, respectively. And t_1 and t_2 are the retention times measured from time of injection to time of elution of peak maximum.

Capacity Factor Calculation (*k'*)

$$k' = \frac{t_1}{t_a} - 1 = \frac{3.0\ \text{min}}{0.8\ \text{min}} - 1 = 3.8$$ USP <621>

Express k' in terms of the amount of mobile phase it takes to elute each compound off the column using the retention volumes

$$k' = \frac{V_1 - Va}{Va} = \frac{3.35cm - 0.70cm}{0.70cm} = 3.8$$

Relative Retention Time (R_R) of Peak 2 with respect to Peak 1

$$R_R = \frac{t_2}{t_1} = \frac{7.0\ \text{min}}{3.0\ \text{min}} = 2.3$$ USP <621>

Resolution between Peak 1 and Peak 2

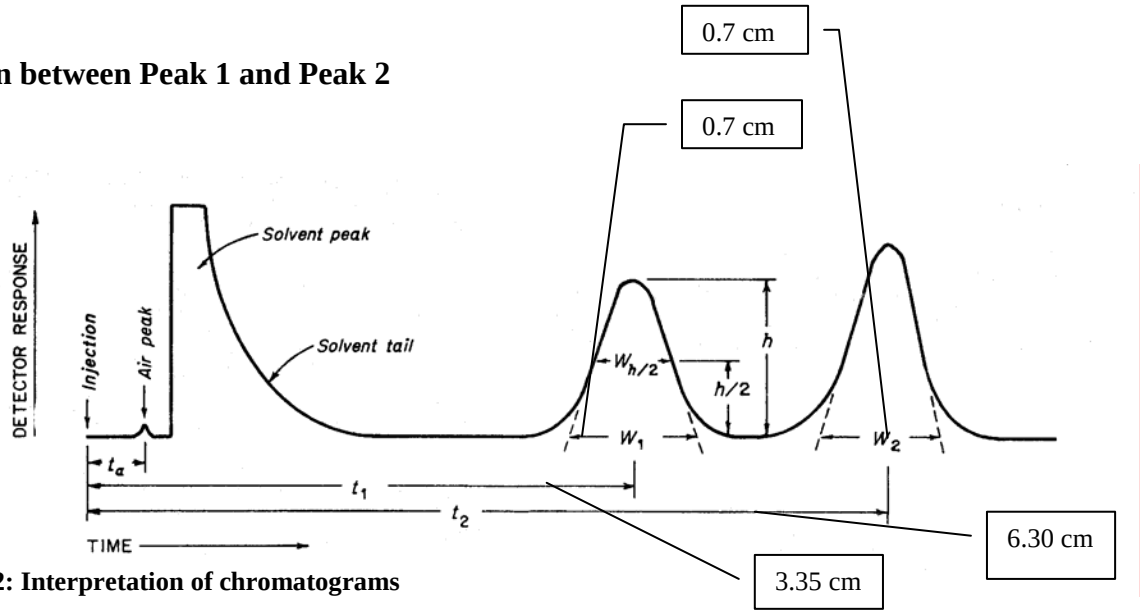

Figure 2: Interpretation of chromatograms

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1}$$ (The retention times and the widths have the same units) USP <621>

$$R = \frac{2(6.30cm - 3.35cm)}{0.7 + 0.7} = 4.2$$

Note: The USP provides another formula to determine resolution and the number of theoretical plates that are convenient when electronic integrators are used. Refer to USP <621>

Tailing Factor (T) Calculation USP <621>

$$T = \frac{W_{5\%}}{2f} = \frac{12.0cm}{5.0cm \times 2} = 1.2$$

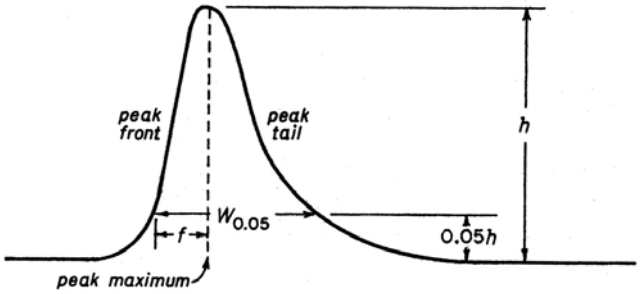

Figure 3: Asymmetrical chromatographic peak

Number of Theoretical Plates (N) USP <621>

Values from Figure 2

$$N = 16\left(\frac{t}{W}\right)^2 = 16\left(\frac{3.35cm}{0.7cm}\right)^2 = 366.4$$

Note: The USP provides another formula to be used with the value of $W_{h/2}$, the peak width at half-height. There is also in the USP another convenient formula to be used when electronic integrators are used.

Calculations using peak areas and peak heights USP <621>

"Peak areas and peak heights are usually proportional to the quantity of compound eluting. These are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs." Ref. " USP <621>.

4. What is the purpose of the internal standard used in exercise 2? Calculate one set of sample results without using the internal standard. Are the results different? If so explain why differences may result.

A major source of error in quantitative comparison is irreproducibility in the amount of sample injected, notably when manual injections are made with a syringe, in the case of GC methods. In these cases, the effects of variability can be minimized by addition of an internal standard. Today's automatic injectors greatly improve the reproducibility of sample injections and reduce the need for internal standards. Ref. USP <621>.

Following, a theoretical calculation example using an internal standard:

Injection	Area of Analyte	Area Internal Standard	Response Ratio (Analyte/Internal STD)
STD	38.14009	35.60928	1.07107
Inj1	37.02482	36.19063	1.02305
Inj2	29.65308	27.93705	1.06142
STD	38.72269	34.96597	1.10744

Average of STD Bracket: 1.07274

Concentration of the STD (C): 10.143 mg in 50 mL volumetric flask: 0.20286 mg/mL
Label Claim of the product: 5 mg
Dilution Factor: 100
Sample weight: 0.36135 mg
Average tablet weight (ATW): 0.09059 mg

To determine the quantity, in mg, of the portions of tablet taken:

$$mg = 100C\left(\frac{R_u}{R_s}\right)\left(\frac{\text{ATW}}{\text{Sample Weight}}\right)$$

Where R_u and R_s are the ratios of the peak responses of the corresponding analyte and internal standard peaks obtained from the Assay preparation and the Standard preparation, respectively.

Example for injection 1

$$mg = 100 \times 0.20286\left(\frac{1.02305}{1.07274}\right)\left(\frac{0.09059}{0.36135}\right) = 4.85mg$$

Percent of label claim: 4.85/5 = 97.0%

Example for injection 2

$$mg = 100 \times 0.20286\left(\frac{1.06142}{1.07274}\right)\left(\frac{0.09059}{0.36135}\right) = 5.03mg$$

Percent of label claim: 5.03/5 = 100.6%

Without using the internal standard…

We used peak responses instead of the ratios:

$$mg = 100 \times 0.20286\left(\frac{37.02482}{38.43139}\right)\left(\frac{0.09059}{0.36135}\right) = 4.90mg$$

Percent of label claim: 4.90/5 = 98.0%

$$mg = 100 \times 0.20286\left(\frac{29.65308}{38.43139}\right)\left(\frac{0.09059}{0.36135}\right) = 3.92mg$$

Percent of label claim: 3.92/5 = 78.4%

THERE IS A DIFFERENCE!
Sample and Standard solutions that do NOT contain an internal standard CANNOT compensate for variations in injection systems, injection volumes and sample/standard solutions.

5. **When and why would someone use gradient elution in HPLC? What is the effect of temperature on HPLC? Would temperature programming such as found in GC be effective for HPLC?** Ref. USP <621>.
   - "The technique of continuously changing the solvent composition during the chromatographic run is called gradient elution or solvent programming. It is sometimes used to chromatograph complex mixtures of components differing greatly in their capacity factors. By increasing the mobile phase strength over time during the chromatographic separation the separation time between components is decreased."
   - "Retention in all LC separations generally decreases with increase in temperature. Another effect is the increase of the column efficiency. At elevated temperatures viscosity of liquids decrease and the diffusion coefficient increase. However, *temperature effects in HPLC are not as significant as in gas chromatography*. Volatile solvents cannot be allowed to rise to higher temperatures, and the high temperature may influence the stability of the attached bonded ligands on the adsorbent surface. The common temperature range for HPLC is from ambient temperature up to 60 or 70 C.
   - Temperature programming is not effective for HPLC.

6. **(To be done at completion of each chromatography sections.). Prepare a chart, comparing and relating each of techniques used in chromatography for the following: column materials, separation theory, phases, equipment needed, ease of use, accuracy, sampling and sampling techniques, automation, accuracy, and quantitation.** See Q & A Attachment A

3.4.2.4(I) Dissolution

1. **What are the parameters that need be checked and corrected before dissolution can be run? Describe what effect each would have on an analysis.**
    - Temperature is to be at 37° C +/- 0.5°C (C (unless otherwise specified).
        - Temperatures higher or lower can effect the rate the product goes into solution
    - Rotation speed of paddle or basket.
        - The faster the speed the faster the product is likely to go into solution.
    - Paddle or basket height or positioning.
        - Both are specified in the USP as 25 +/- 2mm from bottom of paddle/basket to interior vessel bottom. The distance can have a great affect for paddles which relates to the distance from the product. The affect is minimal for the basket where the product is within the basket.
    - Other Physical Parameters (level, wobble, runout, centering).
        - If these parameters are not correct they can seriously after the dissolution rates generally resulting in erroneous results.
    - The vessel also needs to be checked physically for proper dimensions, cleanliness, irregularities and for micro-fractures or scratches in the glass.
        - Improper dimensions and irregularities can cause turbulence and interferences.
    - Each method in the USP is developed using a specified set of parameters. The 'Q' value and general limits of each (extended release) product are established using the parameters listed. If any parameter is not correct then the Dissolution analysis is not valid.

2. **What is the purpose of using two USP calibrators when checking a dissolution system?**
    - Dissolution is very technique-dependant and subject to many variables. Both of the calibrators are used since they detect problems with mechanical calibration, proper de-aeration, temperature control, vibration and hydrodynamic influences.
        - The USP Prednisone Tablets RS calibrators are disintegrating tablets, highly affected by mechanical parameters and vessels.
        - The USP Salicylic Acid Tablets RS calibrators are non-disintegrating tablets, affected by poorly de-aerated media, warped or dirty baskets, and unclean or scratched vessels. They are not as sensitive to mechanical parameters.

3. Exercise 2 demonstrates a profile analysis. When would this technique be used? For what type of products could be technique be used? Look in the USP and list a monograph that uses a profile type analysis. Use this technique to determine the full time range in which a tablet or capsule dissolves. This is frequently used to develop dissolution methods and determine the optimal time that the 'Q' value is established. A modified form of the profile technique is used for controlled release products, where a sample solution is drawn at set times over an extended period. Frequently used for 6, 8, 12, 16 and 24 hr extended release capsules and

tablets. Also, Transdermal Delivery Systems use modified procedures that determine a profile of a product over a period of time. The USP <724> describes several methods and acceptance tables for Extended-Release, Delayed Release and Transdermal Delivery Systems. Products include Clorpheniramine Maleate Extended Release Capsules, Aspirin Extended Release Tablets, and Nicotine Transdermal Systems.

4. Describe the reason and procedure for removing air from the dissolution media? Dissolved gases can cause bubbles to form, which may change the results of the test. Ref. USP <711>. One method of de-aeration is as follows: Heat the medium, while stirring gently to about 41°C. Immediately filter under vacuum using a filter having a porosity of 0.45 um or less. Provide vigorous and continuous stirring of the filtrate under vacuum for 5 minutes. Media should be de-aerated immediately before use. Gently transfer the medium directly to the vessel. Do not introduce air into the medium. Other validated de-aeration techniques for removal of dissolved gases may be used. Ref. USP <711>. There are also commercial apparatus that use a vacuum to draw the medium through a fine pinhole into a large holding vessel.

5. Why not use water for all dissolution media and have constant paddle/basket rotation speed for all determinations? Many drugs are insoluble or have limited solubility in water. Buffers dilute acids and surfactants are routinely used for Dissolution medium. They increase dissolution rates and stabilize the pH of resulting solutions. Test conditions such as pH and surface tension can vary depending on the source of water and may change during the dissolution test itself, due to the influence of the active and inactive ingredients. The paddle/basket speeds can affect the rate of dissolution, generally the faster the speed the faster the dissolution. Methods have been developed to optimize the paddle/basket speeds so that a satisfactory 'Q' value is obtained within a specified time.

6. Can one compare in-vitro dissolution results with in-vivo clinical studies? Explain the answer. Yes. In general an in-vitro, in-vivo (IVIV) correlation has been recognized. It is the goal of the pharmaceutical scientist to find a relationship between an in-vitro characteristic of a dosage form and its in-vivo performance. An effort to connect dissolution and pharmacokinetics is often referred to as "in vitro-in vivo correlation" (IVIVC) analysis. Numerous IVIVC studies can be found in the literature. Controlled release products, rather than immediate release products, are the focused in the IVIVC literature. Since only products with dissolution rate-limited absorption (and with complete complete absorption) can be expected to exhibit a slope of one and zero intercept (Y=mX) mX). The relationship is not as well defined for immediate release products, but depending on the in-vitro release rate and the in-vivo adsorption rate, the two methods are generally recognized as satisfactory for comparison.

Attachment A. I. Column Chromatography, Question 2

Technique	TLC	Column		GC	HPLC
Separation Technique	Adsorption, partition or a combination of both effects	Adsorption and Partition		Partition between gas phase and stationary phase	Partition, adsorption or ion-exchange
Phases	***Stationary*** Adsorbent – thin uniform layer or dry, finely powdered material such as silica gel or cellulose applied to a glass, plastic, or metal sheet or plate. ***Mobile*** Suitable solvent system	*Column Adsorption (CAC)* ***Stationary*** Adsorbent – activated alumina or silica gel as a dry solid or as a slurry ***Mobile*** Suitable solvent	*Column Partition (CPC)* ***Stationary*** Solvent adsorbed on a solid support ***Mobile*** Suitable solvent	***Stationary*** Solid or immobilized liquid stationary phase. Liquid phases are found in packed or capillary columns. ***Mobile*** Gaseous mobile phase	***Stationary*** Solid or immobilized liquid stationary phase ***Mobile*** Liquid mobile phase
Equipment Needed	Glass plates, storage rack, adsorbent, spreader, developing chamber, template, micropipette, sprayer and ultraviolet light source.	Chromatographic tube, delivery tube to control the flow rates of solvent and a tamping rod		Carrier gas source, injection port or auto-injectors, column, heated oven compartment, detector, and data handling system	Mobile Solvent, Solvent Delivery System (Pump), Injector (Autoinjector), column, detector, and data handling system
Ease of Use	User Friendly	User Friendly		Moderate to complex depending on Instrumentation	Moderate to complex depending on detectors and data handling system
Accuracy	Used for semi-quantitative or quantitative estimation	Used for quantitative analysis with the aid of titrimetric or spectrophotometric determinative step		Reliable quantitative results are obtainable especially with internal standards and using auto-injectors or auto-samplers	Reliable quantitative results are obtainable especially with internal standards and auto-injectors or auto-samplers.
Sampling Tech-niques	Apply the Test and Standard Solution as directed in the individual monograph and allow drying	CAC-Compounds are dissolved in a small amount of solvent and added to the top of the column. CPC-A solution of the sample in a small volume of the mobile phase is added to the top of the column or a solution of the sample in a small volume of the immobile phase is mixed with the solid support and transferred to the column		Compound of interest is to be volatile and thermally stable when heated. The test mixture either in a solution or as a gas may be injected directly into the column	Compounds are dissolved in a suitable solvent. This technique allows for thermally unstable and non-volatile compounds to be chromatographed.
Auto-mation	Not normally Multi spotting equipment is available.	None		Auto-injectors, auto-samplers	Auto-injectors, auto-samplers
Cost	Moderately Inexpensive (<$500)	Inexpensive (<$100)		Expensive (>15K +)	Expensive (>20K +)